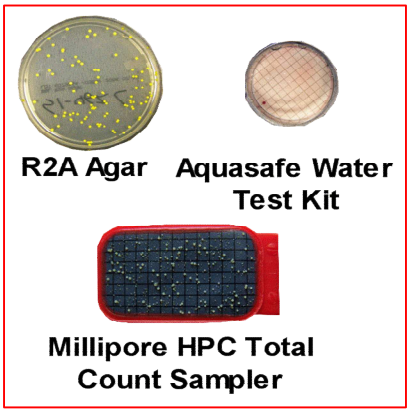
Monitoring Dental Unit Water (7/07)

Cohen ME, Harte JA, Stone ME, O'Connor KH, Coen ML, Cullum ME. Statistical modeling of dental-unit-water bacterial test kit performance. J Clin Dent 2007;18:39–44.

While it is important to monitor dental water quality, it is unclear whether in-office test kits provide bacterial counts comparable to Method 9215C using R2A agar which is considered to be the gold standard method. Studies were conducted on specimens with known bacterial concentrations and from dental units to evaluate test kit accuracy across a range of bacterial types and loads. Colony forming units (CFU) were counted for samples from each source, using R2A agar and two types of test kits, and conformity to Poisson distribution expectations was evaluated. Poisson regression was used to test for effects of source and device, and to estimate rate ratios for kits relative to R2A. For all devices, distributions were Poisson for low CFU/mL when only beige-pigmented bacteria were considered. For higher counts, R2A remained Poisson, but kits exhibited over-dispersion. Both kits undercounted relative to R2A, but the degree of undercounting was reasonably stable. Kits did not grow pink-pigmented bacteria from dental-unit water identified as *Methylobacterium rhodesianum*. **Only one of the test kits provided results with adequate reliability at higher bacterial concentrations. Undercount bias could be estimated for this device and used to adjust test kit results. Insensitivity to *Methylobacteria* spp. is problematic.**



DECS Comment: In USAF dental facilities the recommendation is to use dental water that meets the EPA regulatory standards for drinking water (i.e., 500 CFU/mL of heterotrophic water bacteria) for non-surgical dental treatment. Acceptable dental unit water monitoring methods include submitting water samples to a microbiology lab or to the bioenvironmental engineering section for evaluation using Method 9215C with R2A agar or by using an in-office self-contained commercial system that is equivalent to method 9215. While in-office testing is more practical (e.g., does not require outside laboratory support, less time consuming, more cost effective, more likely to increase compliance) there is some controversy as to whether test kits provide bacterial estimates consistent with laboratory findings. In studies comparing the in-office test kits to the gold standard using R2A agar one study found that the in-office kits were relatively accurate, while in another study they undercounted.[1,2] Another study found high correlations between the kits and R2A agar, but the kits tended to undercount, but not by more than 0.5 log units.[3] A fourth study showed that some dental unit water bacteria do not grow on the in-office test kit media.[4]

The current study compared two commercially available in-office water test kits-the Millipore HPC Total Count Sampler (Millipore, Inc., Bedford, MA) and the Aquasafe Water Test Kit (Pall Medical, Ann Arbor, MI)-relative to Method 9215C (spread plate method) using R2A agar. Both kits undercounted relative to R2A, but the degree of undercounting was reasonably stable. Kits did not grow pink-pigmented bacteria from dental-unit water identified as *Methylobacterium rhodesianum*. Karpay and colleagues reported similar findings when studying the Millipore test kit.[1] *Methylobacteria* spp. has been identified as an opportunistic pathogen in immunocompromised individuals. While it is not recommended to routinely test for specific organisms except when investigating a suspected waterborne disease outbreak, the authors only performed testing to identify the pink-pigmented bacteria because they were noted to be growing on the R2A agar and not on the in-office test kits. This partially explains the reason for the lower counts observed in in-office test kits. As a result, clinicians should be aware that because test kits are insensitive to at least one genus of relevant bacteria, there is some level of unknown false negative risk.

In the present study, the R2A agar plates produced regularly spaced colonies, easily hand-counted without magnification; the Millipore devices yielded evenly spaced colonies but they were smaller and difficult to count without magnification; and colonies on the Aquasafe devices were very small, irregularly distributed, and virtually impossible to count without magnification. As the concentration increased beyond a critical level, the counts tended to decrease on the Millipore devices, probably due to merging of small colonies. This illustrates another possible reason for the inaccuracy noted when using the in-office test kits-the increasingly unreliable segregation of CFU on the smaller test-kit surface areas as the bacterial density increases. Also, the undercounts observed could be the result of the test kits retaining less specimen volume than specified.

The in-office test kits are intended to be used as screening devices when monitoring dental unit water quality. In the present study, only the Millipore test kit provided results with adequate reliability at higher concentrations, and the authors suggested multiplying a Millipore test kit result by a factor of 1.5 to obtain an estimate that is suitable for testing compliance with respect to the 500 CFU/mL count limit (as it would be determined when using R2A agar if pink-pigmented bacteria are not present in the water sample). In other words when using the Millipore HPC Sampler, a count greater than 333 (333 X 1.5=500) would indicate failure with respect to the 500 CFU/mL criterion. Use of the correction factor may provide some assurance that the water quality meets standards.

Further research is needed to determine the accuracy of in-office devices for monitoring dental unit water quality and developing standard techniques to monitor dental unit water bacterial levels. Because one other published study (in addition to the present study) and several abstracts have shown less than favorable results for the Aquasafe water test kit[2,5,6], USAF personnel should seek other water monitoring methods (e.g., Method 9215C using R2A agar, Millipore test kit). If using an in-office test kit, sending dental unit water samples to Bioenvironmental Engineering or a Microbiology lab for evaluation using R2A agar may be indicated periodically (e.g., once a year) or whenever you have any question about the in-office water test results.

References

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